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Dietary Folate Intake and Breast Cancer Risk: Results from the Shanghai Breast Cancer Study[1]

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ABSTRACT

Folate is involved in DNA synthesis, repair, and methylation. It has been hypothesized that high intake of folate may reduce the risk of human cancers, including cancer of the breast. Using data from a population-based case-control study of breast cancer conducted in urban Shanghai during 1996–1998, we evaluated the association of dietary folate intake and breast cancer risk among 1321 cases and 1382 controls, 25–64 years of age, who never drank alcohol regularly or used vitamin supplements. Usual dietary habits were assessed with an in-person, interviewer-administered food frequency questionnaire developed and tested for use in this population. Unconditional logistic regression models were used to calculate odds ratios (ORs) and their 95% confidence intervals (95% CIs) after adjusting for potential confounding factors. Dietary folate intake was inversely associated with breast cancer risk (*P* for trend, 0.05) with an adjusted OR of 0.71 (95% CI, 0.56–0.92) observed among women who were in the highest quintile of intake. The inverse association was stronger after further adjusting for total fruit and vegetable and animal food intakes (OR, 0.62; 95% CI, 0.46–0.82; *P* for trend, 0.01). A more pronounced inverse association between folate intake and breast cancer risk (OR, 0.47; 95% CI, 0.25–0.88; *P* for trend, 0.01) was observed among women who consumed high levels of folate cofactors (methionine, vitamin B_{12}, and vitamin B_6) than those whose intake levels of these nutrients were low. Dietary intake of methionine, vitamin B_{12}, and vitamin B_6 were not independently related to risk of breast cancer after adjusting for confounding factors. Thus, our study adds additional support to the protective role of dietary folate in breast carcinogenesis and suggests further that the effect of folate may be modified by dietary intake of methionine, vitamin B_{12}, and vitamin B_6.

INTRODUCTION

Folate, a B vitamin found naturally in many food sources, particularly in dark green leafy vegetables, is essential for regenerating methionine, the methyl donor for DNA methylation, and for producing the purines and pyrimidine thymidylate required for DNA synthesis and repair. The role of folate is well documented in the prevention of neural tube defects and is increasingly studied in relation to cardiovascular disease risk (1, 2). Evidence for its potential role in carcinogenesis is encouraging, although the effect is less well studied (3). Several studies have examined the association of dietary folate with colorectal neoplasms (4–21), and most of them reported a statistically significant inverse association. However, only a few studies have examined the association of dietary folate intake with breast cancer risk, and the results from these studies have been inconsistent (22–28). With the exception of only one study (26), the observed inverse association was restricted in subgroups of women, and many of the reported associations were not statistically significant. One study found that folate intake was related to a reduced risk of breast cancer only among premenopausal women (24), whereas others found that the inverse association was restricted to postmenopausal women (23, 27), particularly those who drank alcohol regularly. One nested case-control study, using stored serum samples, found no association between serum folate and breast cancer risk (29). Almost all of the previous studies were conducted in predominantly Caucasian North American populations.

Although two previous studies have evaluated folate according to methionine intake (27, 28), no previous study has evaluated the potential modifying effects of vitamin B_{12} and vitamin B_6. All of these nutrients have important roles in folate metabolism. The methyl group for homocysteine remethylation to methionine is donated by folate. Vitamins B_6 and B_{12} are obligate cofactors; vitamin B_6 is a coenzyme for the conversion to the form of folate used for DNA synthesis or methylation, and vitamin B_{12} facilitates the methyl transfer to homocysteine. Therefore, it is conceivable that a high intake of these folate cofactors and/or their interaction with folate may reduce breast cancer risk. The only published study examining these cofactors found an inverse association between serum B_{12} and homocysteine and breast cancer risk (29).

Since 1998, folic acid fortification of cereal grain foods has been mandated in the United States (30); prior to this time, fortification was optional and not uniformly implemented. Therefore, studies of folate intake conducted in North American populations during the past decade may have been limited by the lack of an appropriate food composition database and, thus, by an inability to assess usual folate intake. Some previous studies were also not able to account for intake of folic acid, a more readily bioavailable form of folate than folate in food and present in vitamin supplements; this may have resulted in misclassifying the intake for some subjects.

To evaluate the association between dietary folate intake and breast cancer risk, we analyzed data from a large population-based case-control study conducted in Shanghai, China. Unlike their Western counterparts, most Chinese women have diets composed mainly of unprocessed and unfortified foods, and they rarely take vitamin supplements; this facilitates assessment of folate intake and reduces potential misclassification. Also few women drink regularly. These unique features provide us a better opportunity to evaluate the hypotheses relating dietary folate intake to breast cancer risk than would be available in the United States and other Western countries.

SUBJECTS AND METHODS

Subject Recruitment. The Shanghai Breast Cancer Study is a population-based case-control study conducted in urban Shanghai, China during 1996–1998. Detailed study methods have been published elsewhere (31). Briefly, cases were identified using a rapid-case ascertainment system, supplemented by the Shanghai Cancer Registry. All incident breast cancer cases, newly diagnosed during the study period, and meeting the following criteria were eligible for this study: 25–64 years of age, resident of urban Shanghai, no previous history of any cancer, and alive at the time of interview. A total of 1602 eligible breast cancer cases were identified during the study period, of

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which 1459 cases (91.1%) completed in-person interviews. The major reasons for nonparticipation were refusal (109 cases, 6.8%), death prior to interview (17 cases, 1.1%), and inability to locate (17 cases, 1.1%). Written informed consent was obtained from all subjects, and relevant committees for the use of human subjects in research approved the study protocol. Two senior pathologists confirmed all diagnoses.

Controls had inclusion criteria identical to those of the cases with the exception of a breast cancer diagnosis. Controls were randomly selected and were frequency matched on age (5-year intervals) to the expected age distribution of the cases in a 1:1 ratio. Controls were selected using the Shanghai Resident Registry, a population registry containing address and demographic information for all residents of urban Shanghai. Of the 1724 eligible controls, 1556 (90.3%) completed in-person interviews. The remaining women were not included in the study either because of refusal (166 controls, 9.6%) or death prior to interview (2 controls, 0.1%).

Data Collection and Nutrient Estimation. All subjects completed an in-person interview that used a structured questionnaire and included anthropometric measurements. Dietary intakes were assessed using a 76-item food frequency questionnaire that ascertained mostly single food items as raw ingredients. Only 6 of the food items were processed foods (tofu, fried tofu, breads, noodles, candy, and desserts). The food frequency questionnaire was designed to capture >85% of foods consumed by Shanghai residents and to assess usual diet over the past 5 years, ignoring any recent change. Typically studies conducted in the United States only estimate intake over the past 12 months, which could be problematic, particularly when cases are interviewed several months after cancer diagnosis. Each subject was asked about the frequency with which she ate a specific food (daily, weekly, monthly, yearly, or never), followed by a question on the raw amount typically eaten; *i.e.*, how many lians (1 lian is 50 g) or jins (1 jin is 500 g). For all seasonal foods, subjects were asked to describe their consumption when the food was available on the market. Only 306 cases and controls reported drinking alcoholic beverages regularly (n = 122; 4.1%) or regularly using a vitamin B or multivitamin supplement (n = 188; 6.2%). Alcohol use was missing for 6 (0.2%) subjects. Because alcohol consumption may increase folate requirements and because the data on the folate content of vitamins were not available, all analyses were limited to the 1321 (90.5%) cases and 1382 (88.8%) controls who were known not to consume alcohol and not to take vitamin supplements. Total dietary intakes of folate, methionine, vitamin B_{12}, and vitamin B_6 were calculated by summing the product of the micronutrient content of each food item, usual portion consumed, and frequency of consumption. For each food item listed in the food frequency questionnaire, an identical (82%) or equivalent (17%) item from the food composition database of the USDA was identified, and the values for folate, methionine, vitamin B_{12}, and vitamin B_6 were used in the estimate of intake levels of these nutrients. One minor food item (conch) was excluded because of no comparable item in the USDA food database.

Data Analysis. Quintile distributions among controls were used to categorize all dietary intake variables. Pearson correlation coefficients were used to measure the correlation between all dietary intake variables and daily fruit and vegetable and animal intakes. ORs[3] were used to measure the association of breast cancer risk with dietary intake. Unconditional logistic regression models were used to obtain maximum likelihood estimates of the ORs and their 95% CIs, after adjusting for potential confounding variables. Risk factors identified previously as having an independent association with breast cancer in this population were controlled in all models (31). These included breast cancer in a first-degree relative, history of fibroadenoma, age at menarche, age at first live birth, age at menopause, BMI, physical activity, and menopausal status. Models also controlled for age, education, and household income. Age was included as a continuous variable throughout, and categorical variables were treated as indicator variables in the model. Energy adjustment was performed using the residual method (32). Other dietary factors were also controlled in some analyses. Tests for trend were performed by entering categorical variables as continuous. The folate cofactor score was calculated by summing the ranks in the tertiles for each of methionine, vitamin B_6, and vitamin B_{12}. Analyses stratified by each folate cofactor and the cofactor score were used to evaluate their potential modifying effects. Tests for multiplicative interaction were done by including a multiplicative variable between two variables in the logistic model. All statistical tests were based on two-sided probabilities using SAS, version 8.0 (SAS Institute, Inc., Cary, NC).

RESULTS

Comparisons between cases and controls on selected demographic factors and established breast cancer risk factors are presented in Table 1. Results were very similar between the total sample and among the subset used in the folate analysis (data not shown). Compared with controls, cases had higher education, an earlier age at menarche, a later age at first live birth, a later age at menopause, and a higher daily intake of animal foods. Cases were more likely to have a higher BMI or waist:hip ratio, a family history of breast cancer among first-degree relatives, a history of fibroadenoma, and were less likely to exercise regularly than controls.

Mean and median dietary intake values for total energy, folate, and folate cofactors (methionine, vitamin B_{12}, and vitamin B_6) among controls are presented in Table 2. Average intake according to plant and animal source is also presented. Plant foods were the major source for folate (88%) and vitamin B_6 (72%). Animal foods were the major source of vitamin B_{12} (99%). Methionine was obtained approximately equally from both plant and animal foods. Daily intake of fruits and vegetables was moderately correlated with total folate (r = 0.56) and methionine (r = 0.40) and closely correlated with total B_6 intake (r = 0.75). Daily intake of animal foods was closely correlated with methionine (r = 0.81) and vitamin B_6 (r = 0.62) and moderately associated with folate (r = 0.36) and vitamin B_{12} (0.40). The correlations between folate and its cofactors were 0.62, 0.24, and 0.73 for methionine, vitamin B_{12}, and vitamin B_6, respectively (data not shown).

As shown in Table 3, breast cancer risk was significantly inversely associated with dietary folate intake (OR for highest *versus* lowest quintile of intake, 0.71; 95% CI, 0.56–0.92; P for trend, 0.05). This

Table 1 *Distribution of cases and controls by descriptive characteristics for a subset of women in the Shanghai Breast Cancer Study who were known not to drink alcohol or take vitamin supplements, 1996–1998*

Subject characteristic	Cases (n = 1321)	Controls (n = 1382)	P^a
Age (yr), mean ± SD	48.1 ± 7.9	46.9 ± 8.7	0.02
Education, %			
No formal education	3.8	5.3	
Elementary school	8.2	8.4	
Middle or high school	75.6	76.7	
College or above	12.5	9.6	0.04
Household income (Yuan), %			
<4000	20.1	19.0	
4000–5999	32.8	32.9	
6000–7999	12.5	13.2	
8000–8999	20.1	23.3	
9000+	14.6	11.5	0.08
Breast cancer in first-degree relative, %	3.6	2.2	0.02
Ever had breast fibroadenoma, %	9.9	4.9	<0.01
Age at menarche (yr)	14.5 ± 1.6	14.7 ± 1.7	<0.01
Ever had a live birth, %	95.2	96.6	0.06
Number of live births, mean ± SD	1.5 ± 0.8	1.5 ± 0.8	0.32
Age at first live birth (yr), mean ± SD	26.8 ± 4.0	26.3 ± 3.8	<0.01
Postmenopausal, %	33.6	34.7	0.54
Age at menopause (yr), mean ± SD	48.0 ± 4.5	47.5 ± 4.6	0.07
Physically active past 10 years, %	17.6	23.5	<0.01
BMI (kg/m^2), mean ± SD	23.5 ± 3.4	23.2 ± 3.3	0.01
Waist:hip ratio, mean ± SD	0.81 ± 0.06	0.80 ± 0.05	<0.01
Daily animal food intake (g), mean ± SD	97.9 ± 50.9	90.1 ± 45.6	<0.01
Daily plant food intake (g), mean ± SD	287 ± 158	286 ± 163	0.92
Daily energy intake (kcal), mean ± SD	1863 ± 458	1840 ± 462	0.22

[a] For χ^2 test (categorical variables) or t test (continuous variables).

[3] The abbreviations used are: OR, odds ratio; CI, confidence interval; BMI, body mass index; THF, tetrahydrofolate; SAM, *S*-adenysyl-methionine; NHS, Nurses' Health Study; USDA, United States Department of Agriculture.

Table 2 *Dietary intake of folate and selected nutrients among controls, Shanghai Breast Cancer Study, 1996–1998*

Dietary factor	Mean ± SD	Median (25th, 75th percentiles)	Correlation coefficients with intake of: Fruits and vegetables	Correlation coefficients with intake of: Animal foods
Folate (μg/day)				
Total	294 ± 172	256 (194, 345)	0.56	0.36
Plant source	259 ± 166	220 (163, 302)	0.55	0.28
Animal source	34 ± 22	31 (19, 44)	0.22	0.70
From plants, %	87 ± 7	88 (83, 92)		
Vitamin B_{12} (μg/day)				
Total	4.59 ± 4.14	2.85 (1.32, 8.47)	0.19	0.40
Plant source	0.20 ± 0.47	0.04 (0.01, 0.19)	0.28	0.08
Animal source	4.39 ± 4.09	2.62 (1.16, 8.36)	0.16	0.39
From animals, %	93 ± 12	99 (93, 100)		
Methionine (g/day)				
Total	1.64 ± 0.56	1.55 (1.27, 1.89)	0.40	0.81
Plant source	0.81 ± 0.27	0.77 (0.65, 0.93)	0.38	0.26
Animal source	0.83 ± 0.42	0.76 (0.52, 1.03)	0.29	0.90
From animals, %	49 ± 13	50 (41, 57)		
Vitamin B_6 (mg/day)				
Total	1.73 ± 0.58	1.64 (1.35, 1.99)	0.75	0.62
Plant source	1.24 ± 0.45	1.16 (0.94, 1.42)	0.83	0.30
Animal source	0.50 ± 0.26	0.45 (0.32, 0.62)	0.23	0.86
From plants, %	72 ± 10	72 (65, 78)		

association was further strengthened after adjusting for fruit and vegetable intake and animal food intake (OR, 0.62; 95% CI, 0.46–0.82; *P* for trend, 0.01). Analyses by food source of folate revealed similar associations for both plant and animal sources. The ORs were 1.00, 0.77, 0.89, 0.80, and 0.67 (*P* for trend, 0.02) with increasing plant source folate intake and 1.00, 0.93, 0.85, 0.70, and 0.66 (*P* for trend, <0.01) with increasing animal source folate intake after adjusting for fruit, vegetable, and animal food intakes. A high intake of methionine (*P* for trend, 0.01) was associated with a 30% increased risk of breast cancer; the positive association disappeared after adjusting for animal food intake. A 40% elevated risk was observed with a high intake of vitamin B_6. Analyses by source of vitamin B_6 showed no association with plant source B_6 (*P* for trend, 0.99) and a positive association with animal source B_6 (*P* for trend, <0.01). Additionally adjusting for animal food intake attenuated the positive association (*P* for trend, 0.89). The ORs for increasing animal source vitamin B_6 were 1.00, 0.98, 1.04, 0.92, and 1.09. No association between vitamin B_{12} and breast cancer risk was observed. Stratified analyses by menopausal status showed that similar patterns of associations existed in both pre- and postmenopausal women, although the inverse association with folate intake was more evident in postmenopausal (*P* for trend, 0.03) than in premenopausal women (*P* for trend, 0.57; data not shown). The adjusted ORs for the highest *versus* the lowest quintile of folate intake were 0.78 (95% CI, 0.56–1.07) in premenopausal women and 0.66 (95% CI, 0.44–0.99) in postmenopausal women.

Stratified analyses were performed to evaluate potential modifying effects of folate cofactors (methionine, vitamin B_{12}, and vitamin B_6) on the association between dietary folate intake and breast cancer risk (Table 4). Among women who consumed a low level of these nutrients, dietary folate was weakly associated with a reduced risk of breast cancer. A striking inverse association was observed for dietary folate intake among those whose intake levels of methionine, vitamin B_{12}, and vitamin B_6 were high. These data suggest an interaction, although tests for multiplicative interaction were not statistically significant. Additional analyses were conducted to evaluate folate intake and breast cancer risk, stratified by dietary sources of methionine and vitamin B_6. The same pattern of associations was observed (data not shown).

DISCUSSION

In this large population-based case-control study, we observed that dietary folate intake was inversely associated with the risk of breast cancer. This inverse association was particularly evident after adjust-

Table 3 *Risk of breast cancer associated with dietary intake of folate, methionine, vitamin B_{12}, and vitamin B_6, Shanghai Breast Cancer Study 1996–1998*

Dietary intake	Adjusted OR (95% CI) by quintile of intake[a]: Q1 (low)	Q2	Q3	Q4	Q5	*P* for trend
Folate						
Cases/controls	320/277	256/276	256/277	275/276	214/276	
OR (95% CI)	1.00 (reference)	0.83 (0.65–1.06)	0.87 (0.67–1.12)	0.93 (0.72–1.19)	0.71 (0.56–0.92)	0.05
OR (95% CI)[b]	1.00 (reference)	0.78 (0.61–1.01)	0.79 (0.61–1.03)	0.85 (0.65–1.11)	0.62 (0.46–0.82)	0.01
Vitamin B_{12}						
Cases/controls	247/277	267/276	282/277	259/276	266/276	
OR (95% CI)	1.00 (reference)	1.21 (0.93–1.56)	1.29 (0.99–1.67)	1.18 (0.92–1.53)	1.18 (0.91–1.52)	0.33
OR (95% CI)[b]	1.00 (reference)	1.11 (0.85–1.44)	1.14 (0.87–1.49)	1.05 (0.80–1.36)	1.01 (0.77–1.32)	0.79
Methionine						
Cases/controls	234/277	245/276	234/277	304/276	298/276	
OR (95% CI)	1.00 (reference)	1.08 (0.84–1.40)	1.03 (0.80–1.34)	1.34 (1.04–1.73)	1.31 (1.02–1.68)	0.01
OR (95% CI)[b]	1.00 (reference)	0.95 (0.73–1.24)	0.84 (0.63–1.12)	1.00 (0.74–1.36)	0.79 (0.54–1.16)	0.47
Vitamin B_6						
Cases/controls	219/277	268/276	268/277	270/276	296/276	
OR (95% CI)	1.00 (reference)	1.27 (0.98–1.64)	1.26 (0.97–1.62)	1.36 (1.05–1.76)	1.44 (1.12–1.85)	0.01
OR (95% CI)[b]	1.00 (reference)	1.26 (0.97–1.65)	1.22 (0.92–1.63)	1.35 (0.99–1.84)	1.46 (1.01–2.13)	0.07

[a] All ORs and 95% CI were calculated in a logistic regression model adjusted for total energy, age, education, family history of breast cancer, personal history of fibroadenoma, age at menarche, parity, age at first live birth, menopausal status, age at menopause, physical activity, and waist:hip ratio. Quintile cutpoints were based on residual energy-adjusted intake among controls.

[b] Additionally adjusted for total fruit and vegetable intake and total animal food intake.

Table 4 *Risk of breast cancer associated with dietary folate intake by intake of methionine, vitamin B_{12}, and vitamin B_6, Shanghai Breast Cancer Study, 1996–1998*

Dietary intake	Adjusted OR (95%) CI by quintile of folate intake[a]					*P* for trend
	Q1 (low)	Q2	Q3	Q4	Q5	
Methionine tertile						
T1 (low)	165/162	80/105	55/75	69/64	42/55	
	1.00 (ref)	0.75 (0.51–1.10)	0.75 (0.48–1.17)	1.10 (0.72–1.70)	0.83 (0.51–1.32)	0.81
T2	76/63	86/107	103/115	90/110	46/65	
	1.00 (ref)	0.66 (0.41–1.06)	0.73 (0.46–1.17)	0.71 (0.44–1.14)	0.57 (0.34–0.98)	0.11
T3	79/52	90/64	98/87	116/102	116/156	
	1.00 (ref)	0.95 (0.58–1.55)	0.82 (0.50–1.32)	0.81 (0.51–1.30)	0.57 (0.37–0.89)	<0.01
			P for interaction = 0.15			
Vitamin B_6 tertile						
T1 (low)	204/205	98/129	55/71	27/37	15/19	
	1.00 (ref)	0.77 (0.54–1.11)	0.81 (0.52–1.27)	0.71 (0.41–1.24)	0.81 (0.39–1.68)	0.19
T2	69/51	113/108	125/123	113/117	34/61	
	1.00 (ref)	0.77 (0.48–1.24)	0.77 (0.48–1.25)	0.73 (0.45–1.18)	0.43 (0.24–0.77)	0.01
T3	47/21	45/39	76/83	135/122	165/196	
	1.00 (ref)	0.51 (0.25–1.04)	0.47 (0.25–0.89)	0.57 (0.31–1.85)	0.41 (0.23–0.73)	0.01
			P for interaction = 0.26			
Vitamin B_{12} tertile						
T1 (low)	149/139	75/104	64/73	78/72	59/73	
	1.00 (ref)	0.69 (0.46–1.02)	0.85 (0.55–1.31)	1.04 (0.68–1.59)	0.78 (0.50–1.20)	0.69
T2	65/61	94/94	113/98	106/119	77/88	
	1.00 (ref)	0.97 (0.60–1.56)	1.18 (0.73–1.90)	0.88 (0.55–1.41)	0.83 (0.51–1.35)	0.31
T3	106/77	87/78	79/106	91/85	78/115	
	1.00 (ref)	0.90 (0.57–1.42)	0.63 (0.41–0.99)	0.91 (0.58–1.44)	0.53 (0.35–0.82)	0.01
			P for interaction = 0.14			
Folate cofactor score						
3–4 (low)	135/141	58/85	32/48	39/34	13/20	
	1.00 (ref)	0.69 (0.44–1.07)	0.66 (0.38–1.15)	1.10 (0.63–1.91)	0.75 (0.35–1.62)	0.62
5–7	148/114	145/156	158/151	154/163	92/118	
	1.00 (ref)	0.74 (0.52–1.06)	0.93 (0.65–1.33)	0.83 (0.58–1.19)	0.62 (0.42–0.91)	0.07
8–9	37/21	50/34	64/77	84/78	108/137	
	1.00 (ref)	0.85 (0.41–1.77)	0.50 (0.26–0.98)	0.65 (0.33–1.26)	0.47 (0.25–0.88)	0.01
			P for interaction = 0.49			

[a] All ORs are calculated from a logistic regression model and adjusted for total energy, age, education, family history of breast cancer, personal history of fibroadenoma, age at menarche, parity, age at first live birth, menopausal status, age at menopause, physical activity, and waist:hip ratio. Quintile cutpoints were based on residual energy-adjusted intake among controls.

ment for fruits and vegetables and among women whose usual diets contained a high level of methionine, vitamin B_{12}, or vitamin B_6. These findings are consistent with data from animal and *in vitro* studies implicating a potential role of folate in cancer prevention (33–38).

There are several possible mechanisms by which folate deficiency may contribute to the carcinogenic process:

(*a*) Folate deficiency may lead to decreased 5-methyl THF, which would result in decreased levels of SAM and global hypomethylation of DNA (39–41). Methylation is important for modulating gene expression, and hypomethylation has been observed to increase mutation rates via genomic instability (42–46).

(*b*) A second proposed mechanism is an increase in DNA replication errors. When folate levels are low, SAM formation may take precedence over thymidylate synthesis, resulting in a low thymidylate level and increasing misincorporation of uracil into DNA with resulting higher levels of chromosomal breaks (33, 39, 40, 47).

(*c*) Although somewhat contrary, folate deficiency also appears to be associated with hypermethylation of the CpG islands in the promoter region of several tumor suppressor genes or DNA repair genes, reducing their expression (40, 48). Hypermethylation of these genes is associated with the development of cancer (48–51).

Although folate intake has been investigated extensively in relation to colon cancer risk, only a few epidemiological studies have been conducted to evaluate its association with breast cancer risk. Our findings of an overall inverse association of folate intake with breast cancer risk are supported, in general, by three previous case-control studies (23, 24, 26). Of these, two were conducted in Western New York, United States, one among postmenopausal women and the other among premenopausal women, in which high folate intake was associated with a statistically nonsignificant 30% reduction in risk postmenopausally and a statistically significant 50% reduction in risk premenopausally (23, 24). The third study, conducted in Uruguay, also found a statistically nonsignificant 30% reduction in risk (26). A 50% reduction in breast cancer risk associated with folate intake was observed among high consumers of alcohol in the NHS, although the relationship was not observed in the total sample (27). On the other hand, two previous studies reported no association between folate intake and breast cancer risk (22, 25). These two studies, however, may have suffered from recall bias or a small sample size.

In our study, the inverse association appeared to be particularly strong among those who consumed a high level of methionine, vitamin B_{12}, or vitamin B_6, even in the presence of no strong overall associations between these cofactors and breast cancer risk. This suggests that the cofactors may be important through their respective roles in folate metabolism; if the inverse association we observed is mostly attributable to folate, then it is expected that an inverse or no association between the cofactors and breast cancer risk would be observed as in this study. Potential modifying effects of vitamin B_6 or B_{12} on the association between dietary folate and risk have not been evaluated in previous studies of breast cancer. Only two previous studies have examined the association of folate intake and breast cancer risk according to methionine intake level (27, 28). In a case-cohort study conducted in Canada, no modifying effect of methionine was observed (28). That study did not collect data on the folate content of multivitamins; therefore, intakes may have been underestimated for vitamin users. Results from the NHS cohort indicate no overall association between folate intake and breast cancer risk. An inverse association was observed among those who had a low methionine intake (27). However, the NHS differs from ours in that a large proportion of those participants were vitamin users, and the methionine levels in our population were lower than in the NHS. Specifically, the upper cutpoint for the lowest NHS quintile was close to the median intake level in our study. It is possible that we did not observe

a folate association among those with the lowest intake level because for these subjects folate cannot compensate for their methionine deficiency. It can also be speculated that because animal sources are substantial contributors to methionine intake in Western populations, some factor associated with animal food intake, such as red meat intake, may have masked or surpassed the folate association in the NHS.

Vitamin B_{12} and vitamin B_6 are involved in the metabolism of folate, which may explain the potential modifying effect of these nutrients on the association of folate with breast cancer risk. Vitamin B_{12} is a cofactor for the methyl transfer from 5-methyl THF to homocysteine to form methionine and THF. In conditions of low vitamin B_{12} supply, the transfer of methyl groups may be reduced, thus affecting DNA methylation, synthesis, and repair. Vitamin B_6 is a coenzyme for homocysteine catabolism and for the acquisition of one-carbon units from serine to form 5,10-methylene THF, the folate that is used for DNA synthesis or for 5-methyl THF formation. In the presence of low vitamin B_6 supply, the conversion of THF to 5,10-methylene THF may be reduced which may, in turn, affect DNA synthesis or DNA methylation. Similarly, SAM inhibits methylenetetrahydrofolate reductase so that in the presence of adequate methionine, more folate may be available for use in thymidylate synthesis, reducing the probability of uracil misincorporation.

Because folate data are not available in the Chinese Food Composition Table, we used the USDA food composition data to estimate dietary intake values for Chinese women in Shanghai. Excellent match, however, was achieved between the mostly single foods listed in the food frequency questionnaire used in our study and those from the USDA database. Although it is possible that the absolute level of folate in foods listed in the database and those actually consumed in Shanghai differ because of different growing, shipping, processing, or other practices, this type of misclassification should be nondifferential between cases and controls, leading to attenuation of the true association in most situations. This is also true of any seasonal or other differences of foods within the urban Shanghai food supply. Because food biology and compositions are unlikely to be fundamentally different between foods produced in the United States and China and we are interested primarily in the relative intake of foods and nutrients, the estimates obtained in our study should be a relatively accurate reflection of folate intake in our study participants. Although we could not directly examine the correlation of folate intake using the USDA and Chinese databases, we did assess the correlation of three other water-soluble vitamins: vitamin C, riboflavin, and niacin. We found excellent correlation; all Pearson correlation coefficients were 0.91 or higher. These data provide additional assurance that the data on folate intake may be appropriate. As in any case-control study, the possibility of potential selection and recall biases cannot be dismissed completely. However, both cases and controls in this study had very high participation rates, thus decreasing the potential influence of selection bias on our results. Over 50% of cases were interviewed within 15 days after diagnosis, and over 80% were interviewed within 4 months of diagnosis, thus reducing potential measurement errors attributable to recent dietary changes after diagnosis. Furthermore, Chinese in Shanghai eat most of their food prepared using basic ingredients, and this behavior facilitates the quantification of usual diet over a longer period of time (5 years; Refs. 52–54) than the 12-month period assessed in typical studies conducted in Western countries. Confounding also may be a concern. However, we have collected extensive information on all known breast cancer risk factors and carefully adjusted for them in all data analyses. The strengths of our study include the very high participation rates and the ability to estimate folate intake in a population who were nonusers of alcohol and vitamin supplements and who mainly consumed unprocessed and unfortified foods.

In summary, our study found evidence of a decreased risk of breast cancer associated with high consumption of folate among women who do not regularly consume alcohol. This relationship was especially apparent among subjects who also consumed higher amounts of methionine, vitamin B_{12}, or vitamin B_6. This study raises the possibility of important nutrient-nutrient interactions in breast carcinogenesis. As the body of literature on the role of polymorphic genes in folate metabolism increases, it is important for future studies to address the relative contributions of these nutrients and genes in breast carcinogenesis.

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